

Supplementary Table 1: Photosynthetic efficiency

Supplementary Table 1. Photosynthetic Efficiency. The photochemical efficiency, Φ_{PSII} , light dependent thermal dissipation component of nonphotochemical quenching, Φ_{NPQ} , and the sum of fluorescence quenching and light-independent thermal dissipation, $\Phi_{\text{f,D}}$, at normal light and moderate light and drought and their respective sums, Σ , were determined at normal light and moderate light and drought. The mean and standard error are shown for the average of two experiments with four plants per line per treatment per experiment (see Figure 2). Asterisks indicate significant difference ($p < 0.05$) between treatments.

	Normal conditions				Moderate light and drought			
	Φ_{PSII}	Φ_{NPQ}	$\Phi_{\text{f,D}}$	Σ	Φ_{PSII}	Φ_{NPQ}	$\Phi_{\text{f,D}}$	Σ
Col-O	0.49±0.03	0.23±0.03	0.28±0.00	1.00	0.40±0.01	0.33±0.04	0.27±0.03	1.00
<i>aox1a-1</i>	0.46±0.03	0.24±0.03	0.30±0.00	1.00	0.23±0.05*	0.47±0.06*	0.29±0.01	1.00
<i>aox1a-2</i>	0.48±0.05	0.22±0.03	0.29±0.02	1.00	0.19±0.03*	0.45±0.05*	0.36±0.02	1.00

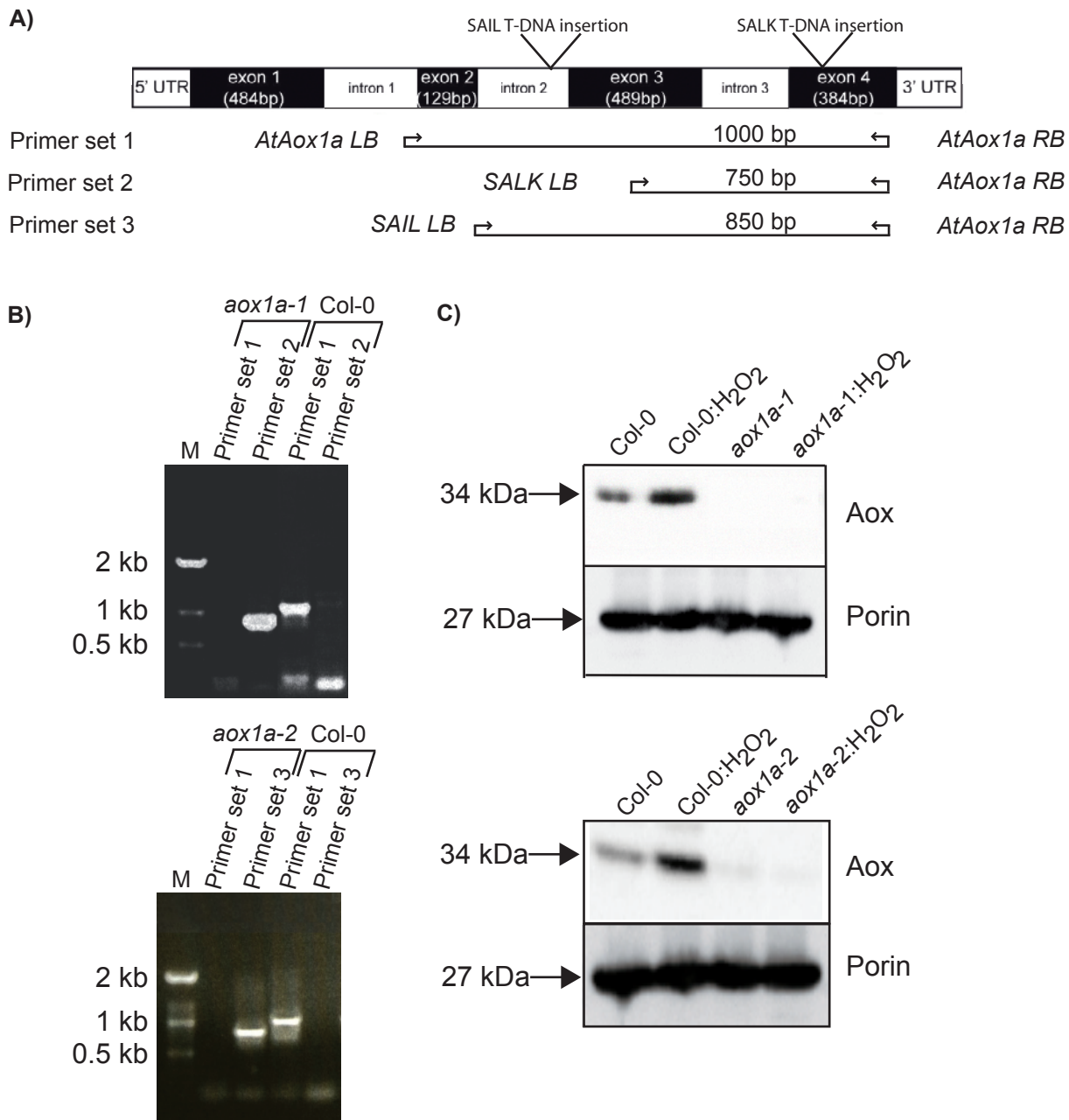
Metabolite Class	Metabolite Name	Genotype	
		<i>aox1a</i>	Col-0
Organic Acids	cis-Sinapinic acid*	1.4	3.2
	Citramalate	1	2.1
	Citrate	2.2	2.1
	Malate	0.7	1.1
	Nicotinate	1.2	1.8
	Succinate	1.4	2.2
Amino Acids	GABA	2.9	4.2
	Glycine	9.7	6.7
	Isoleucine	5.1	2.4
	Leucine	1.7	3
	p-Aminobenzoate	1.4	2.2
	Tyrosine	3	5.2
	Valine	3.3	4.1
	Unknown amino acid [USH: N-Acetylglycine (1TMS), 71]	0.2	0.5
Sugars and Sugar Derivatives	1,6-Anhydro-beta-D-glucose	0.9	0.7
	beta-D-Glc-(1,6)-D-Glc*	34	15
	Fructose	11	2.8
	Galactose	8.1	4.9
	Glucose	8.1	4.9
	Raffinose*	22	14
	Sucrose	1.5	1.6
	Unknown sugar [USH: Arabinofuranose (4TMS), 70]	3.6	1.7
	Unknown sugar [USH: Arabinofuranose (4TMS), 75]	2.8	1.4
	Unknown sugar [USH: beta-D-Glc-(1,4)-D-Glc (8TMS), 85]	2.2	1.5
	Unknown sugar [USH: beta-D-Glc-(1,6)-D-Glc, 76]	1.6	1.1
	Unknown sugar [USH: D-Glycero-D-gulo-Heptose methoxime (6TMS), 79]	1.9	1.5
	Unknown sugar [USH: Digalactosylglycerol (9TMS), 88]	4.2	1.5
	Unknown sugar [USH: D-Ribose methoxime (4TMS), 81]	1.3	1.3
	Unknown sugar [USH: Maltose methoxime (8TMS), 92]	3.6	3.4
	Unknown sugar [USH: Melibiose (8TMS), 76]	12	2.6
	Unknown sugar [USH: Melibiose (8TMS), 83]	46	5.7
	Unknown sugar [USH: Sedoheptulose methoxime (6TMS), 84]	4	3.8
	Unknown sugar [USH: Sedoheptulose methoxime (6TMS), 85]	2.1	1.6
	Unknown sugar [USH: Sedoheptulose methoxime (6TMS), 86]	3.6	3.7
Sugar Acids	Glucuronate	2.4	1.2
	Unknown sugar acid [USH: Galactaric acid (6TMS), 78]	2	2.1
	Unknown sugar acid [USH: Gluconic acid methoxime (5TMS), 89]	2.8	1.7
Polyols	Galactitol	1.1	0.8
	Galactinol*	20	21
	Sorbitol	2.6	1.7
	Threitol	1.5	1.2
Nitrogen-rich	Allantoin	2.1	1.9
Polyamines	Putrescine	1.6	0.9
Phenylpropanoids	Unknown phenylpropanoid [USH: 4-hydroxy-3-methoxyphenethylene glycol (3TMS), 93]	2.5	1.4
Terpenoids	gamma-Tocopherol*	9.8	4.6
	Unknown terpenoid [USH: Phytol (1TMS), 95]	1.4	1.2
Inorganic Acids	Phosphate	1.2	3.9
	Methylphosphate*	1.3	4.2

Supplementary Table 2. Characteristic metabolite response profile of *aox1a* T-DNA insertion lines to moderate light and drought compared with Col-0 response.

Metabolite level changes occurring in response to 3 days of moderate light and drought treatment were detected in Col-0 and two independent *aox1a* T-DNA insertion lines using GC-MS based metabolite profiling (see Materials and Methods). The mean fold change in GC-MS signal levels (across the two independent *aox1a* lines) are shown above for metabolites that responded similarly in both *aox1a* lines. For comparison, the responses of these metabolites in Col-0 are shown in the right-hand column. Metabolite signals that increased significantly ($p < 0.05$, $n = 5$) in response to the stress treatment are highlighted in blue while signals that decreased significantly ($p < 0.05$, $n = 5$) are highlighted in red. Signals that showed no significant change are shown in black. Colour intensities are related to metabolite response intensities with more strongly responsive signals highlighted in brighter tones. * These metabolites were putatively identified by matching against the publicly available Golm Metabolome Database MSRI library (see supplementary information). Compounds contained in square brackets are unknown metabolites with mass-spectral homology to the indicated compound, the number after the compound name being the 'simple' match score reported by AMDIS when searched against the NIST02 MS library.

Supplementary Table 4. List of 17 genes and ubiquitin and primers used for QRT-PCR . The genes tested have been linked to various stress responses. All five genes listed as hallmarks by Gadjev et al., 2006 were analysed, as well as representative genes listed in other studies.

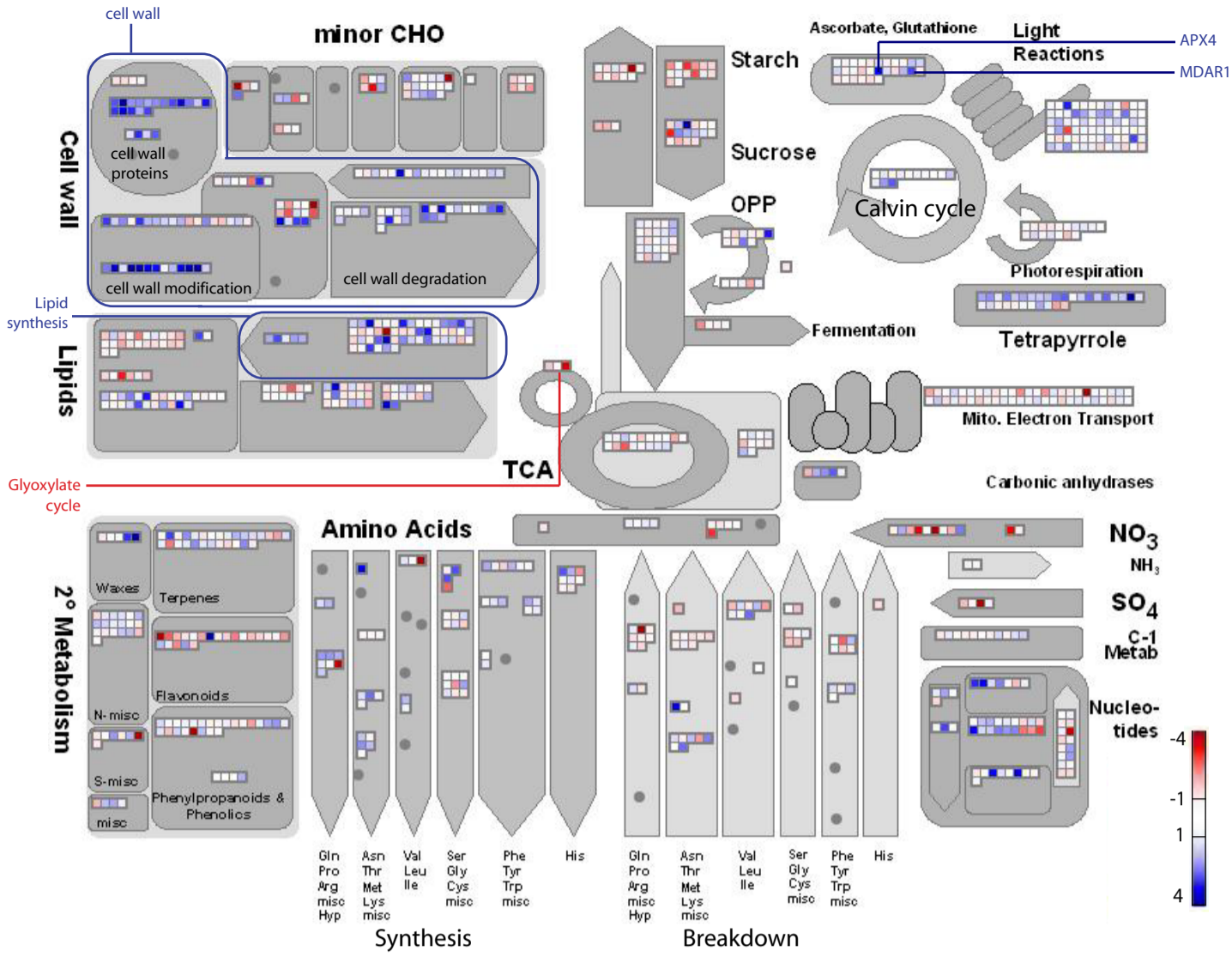
Chromosomal Locus	Gene Description	QRT-PCR primers	Reference
NDB2 (At4g05020)	Mitochondrial NAD(P)H dehydrogenase	Forward: ctgactctctcaaagagtcc Reverse: cgatttgaactctcgatc	Clifton et al., 2006
AOX1a (At3g22370)	Alternative oxidase 1a	Forward: gacggtcctgacggtttcg Reverse: ctctgattcgctcctcctcct	Clifton et al., 2006
UCP (At3g54110)	Uncoupling protein 1	Forward: gaaaactagctgagggtgcg Reverse: acaacgtgtcagtgaaaccc	Clifton et al., 2006; Sweetlove et al. 2007
UPOX (At2g21640)	Unknown Function - <u>Up</u> -regulated by <u>oxidative</u> stress	Forward: cggagaacccgccaaaacc Reverse: gcttctctgcaactgcctc	Clifton et al., 2006; Gadjev et al., 2006
PR1 (At2g14610)	Pathogen related protein 1	Forward: gtgggttagcggagaaggcta Reverse: acttggcacatccgagct	Bechtold et al., 2005
UBC (At5g25760)	Ubiquitin	Forward: ctgcgactcaggaatcttcta Reverse: ttgtgccattgaattgaattgaaccc	Czechowski et al., 2005
At2g43510	Defensin-like protein	Forward: ggctatcgtttccatcttcg Reverse: actcgttaccttgcgcttct	Gadjev et al., 2006
At1g57630	Disease resistance protein RPP1-WsB	Forward: acgcttctcgtgggtgtgt Reverse: cgttgacccgactctttct	Gadjev et al., 2006
At1g27730	Transcription factor - ZAT10	Forward: tttgcctcatgcttctcg Reverse: acactgtagctcaacttctccac	Kilian et al., 2007
At5g51190	AP2 domain transcription factor	Forward: gttcaggctgatgctgtcc Reverse: gctgctccgctccaaaac	Kilian et al., 2007
At5g47230	Ethylene-responsive element-binding factor 5	Forward: tcttcttatcatcttcggatca Reverse: tgcatacggattcagagaaatc	Kilian et al., 2007
At1g19020	Unknown- expressed protein	Forward: ggctgaccagtgaggacaata Reverse: cactccgctgcttctcac	Gadjev et al., 2006
At1g05340	Unknown - expressed protein	Forward: cgtagaagatgagccagtagc Reverse: ggtggtggtgatgtacaggtag	Gadjev et al., 2006
At4g27657	Unknown - expressed protein	Forward: gcaaggcgacagaaatgttat Reverse: aacaggtaggatctaggagga	Kilian et al., 2007
At5g59820	Transcription factor - ZAT12	Forward: cttggaggacacatgagg Reverse: caaagcgcgtgaaccaac	Rizhsky et al., 2004
At4g32320	Cystolic ascorbate peroxidase APX6	Forward: ttgggaagactgtattcagc Reverse: tctgggtcgaaaatccttt	Panchuk et al., 2005
At3g15210	ERF/AP2 transcription factors family member	Forward: atggggatcggtaacgtagg Reverse: cgatctaaacgccgatgc	Yang et al., 2005
At4g27410	NAC transcription factor induced in response to dessication	Forward: gcacgagatcgcttaatagaaca Reverse: cgacacaacaccaatcatc	Lee et al., 2006



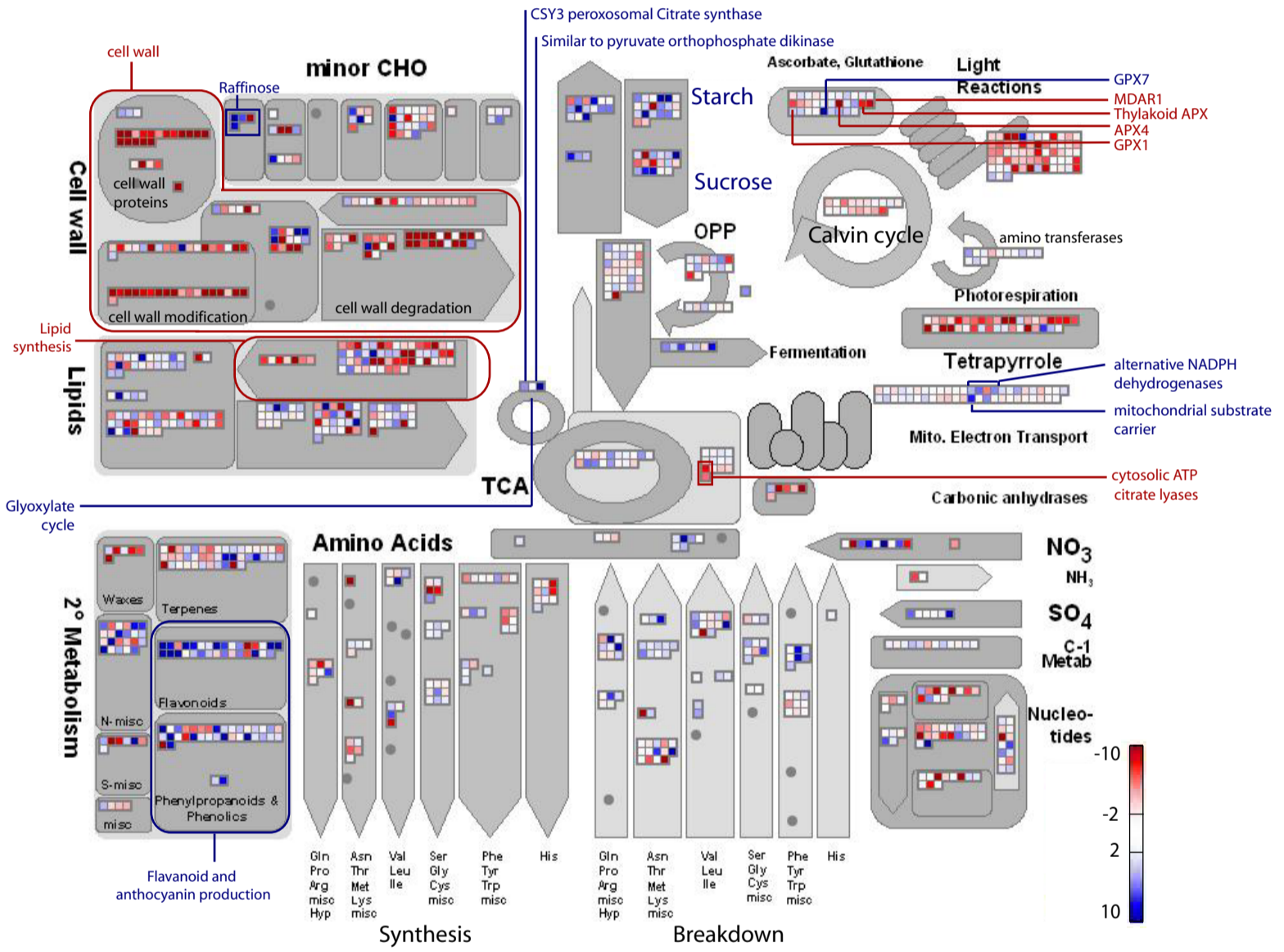
Supplementary Figure 1. Characterisation of the insertional lines inactivating *AOX1a*.

A) A diagrammatic representation of the *AOX1a* gene structure (At3g22370) and the position of the primers used to determine if a T-DNA insert was present. B) RT-PCR with different primer sets to determine if a T-DNA insert was present in the gene; the failure to produce a fragment using primer set 1 in both insertional lines, that were designed to amplify a fragment if the gene is uninterrupted, and the production of a fragment using primers to the left border of the T-DNA (primers sets 2 and 3) indicates that both lines are homozygous knock-out lines. The two lines were designated *aox1a-1* and *aox1a-2*. C) Western blot analysis of mitochondria purified from wild-type Arabidopsis plants (Col-0) and *AOX1a* knock out lines (*aox1a-1* and *aox1a-2*) were probed with antibodies to the alternative oxidase and porin. The lack of a cross-reacting band when probed with antibodies to AOX in the *aox1a-1* and *aox1a-2* lines confirmed that these plants contained an inactivated *AOX1a* gene. Furthermore, treatment of leaf samples with H₂O₂ caused a detectable increase in the band that cross reacted with the AOX antibody in mitochondria isolated from Col-0 plants, but no cross reacting band was present in the *aox1a-1* and *aox1a-2* lines.

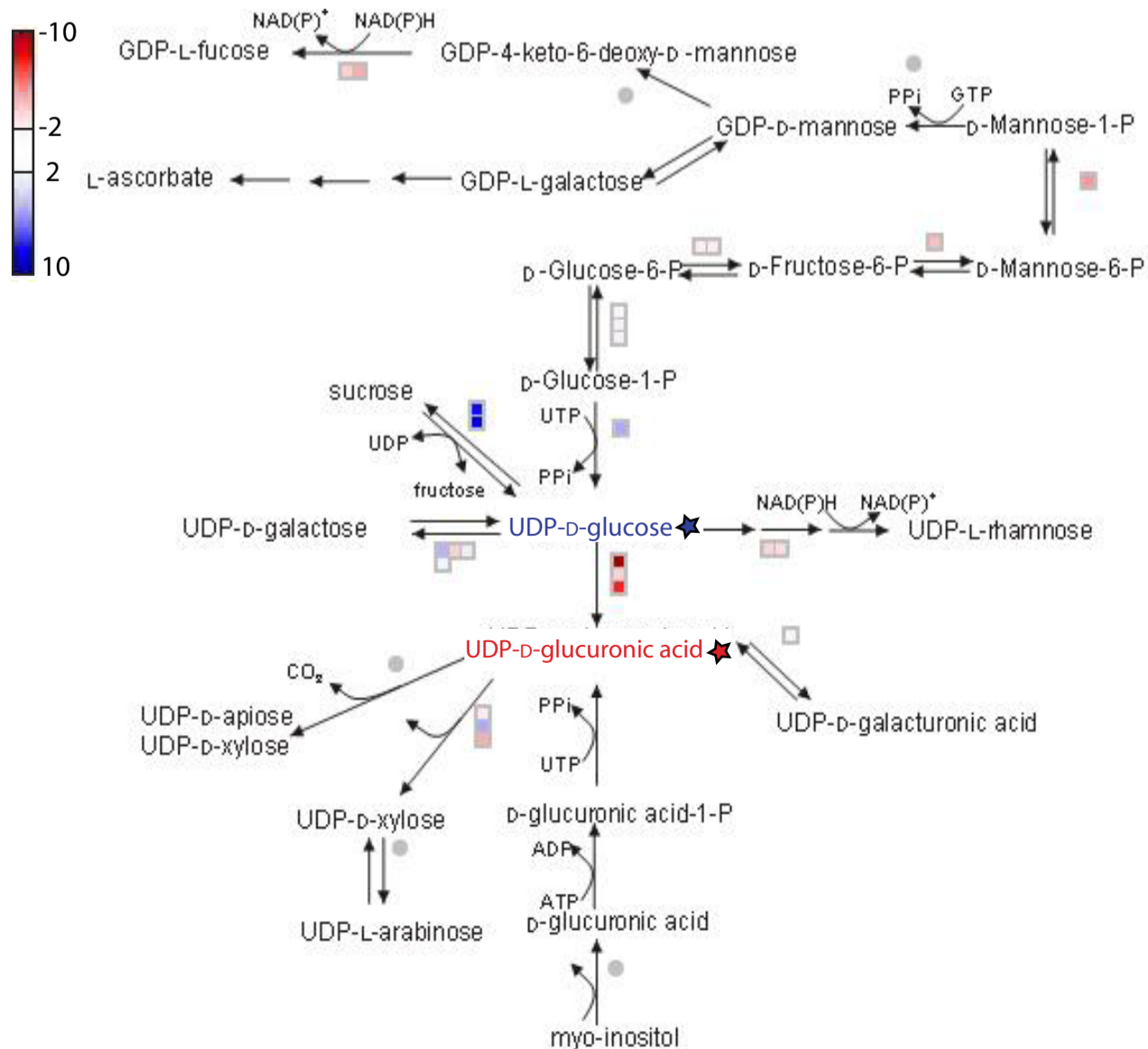
A) Col-0 normal conditions vs *aox1a* normal conditions



B) *aox1a* normal conditions vs *aox1a* moderate light and drought



C) Cell wall Precursors

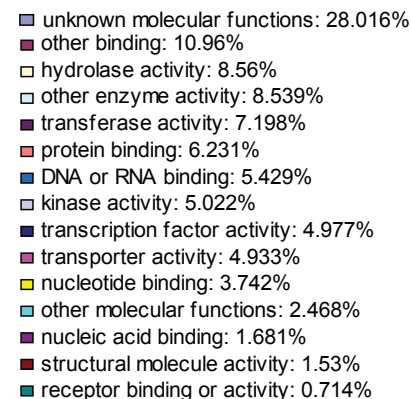
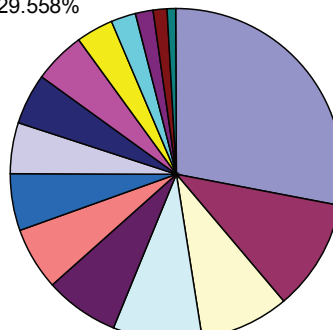
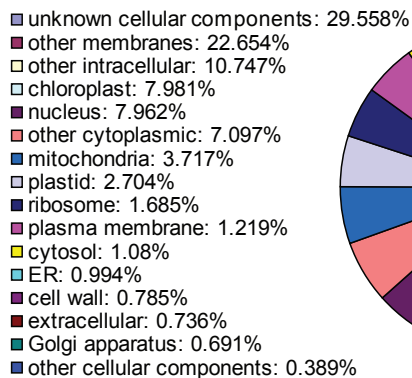
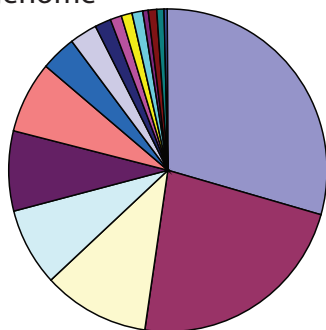


Supplementary Figure 2. Overview of changes in transcript abundance associated with primary metabolism. Transcripts associated with primary metabolism, which are significantly changing after false discovery rate (FDR) correction between A) *aox1a* and Col-0 under normal conditions and. B) *aox1a* normal conditions compared to *aox1a* under moderate light and drought treatment. Changes are displayed using the MapMan software (<http://gabi.rzpd.de/projects/MapMan/>). The abundance ratio or fold-change is shown by the colour scale, red indicating a decrease and blue indicating an increase in transcript abundance. Key genes and areas are highlighted in red and blue based on direction of transcript abundance changes. C) Significant changes in transcript abundance after FDR correction for genes encoding proteins involved in cell wall precursor synthesis pathways in *aox1a* normal conditions versus *aox1a* moderate light and drought treated. Pathway was generated using the MapMan software (<http://gabi.rzpd.de/projects/MapMan/>). The color scale indicates abundance ratios; red indicates a decrease in abundance while blue indicates an increase. Significantly changing metabolites are also indicated as blue (increased) and red (decreased) stars.

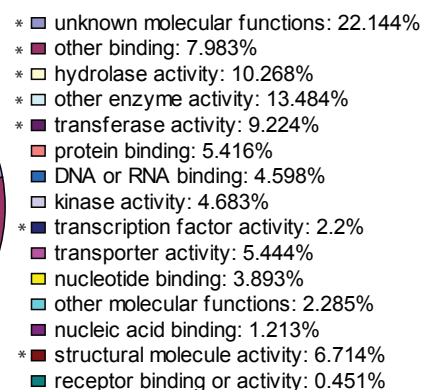
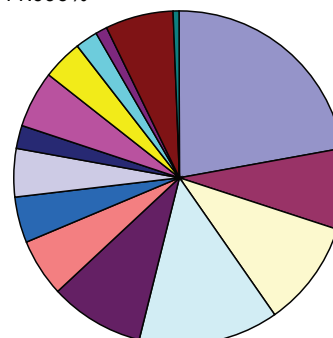
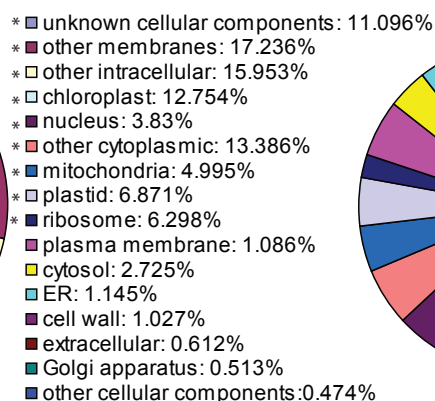
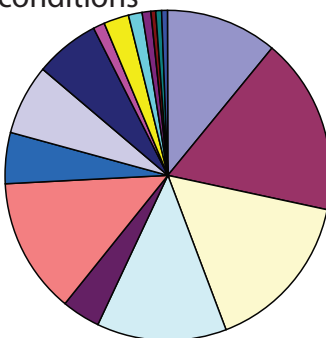
Component

Function

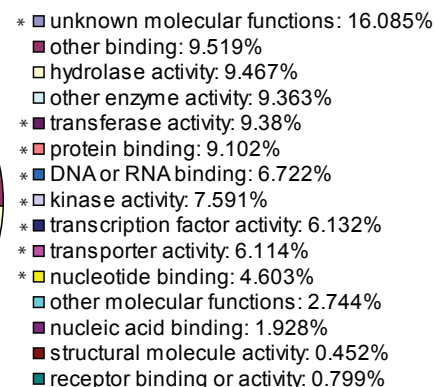
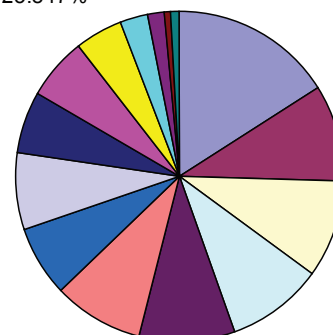
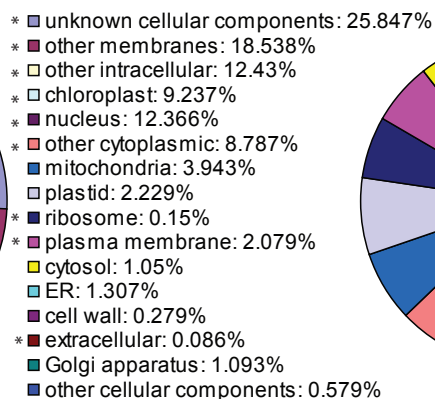
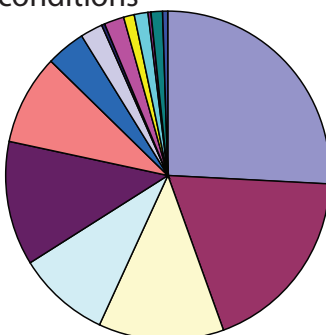
whole genome



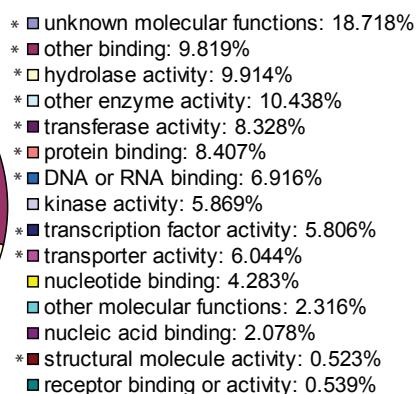
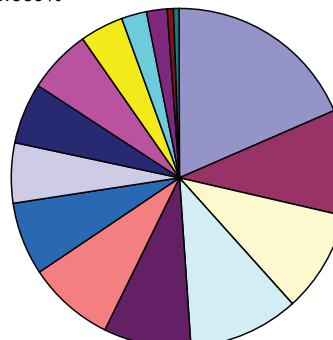
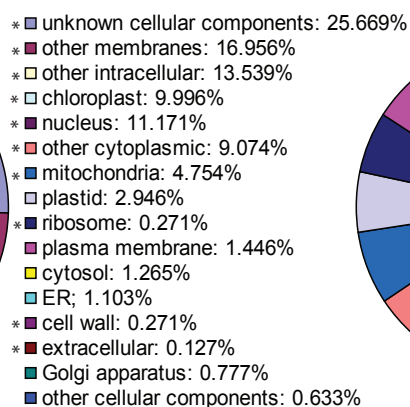
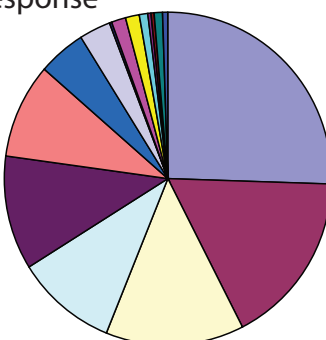
Upregulated under normal conditions



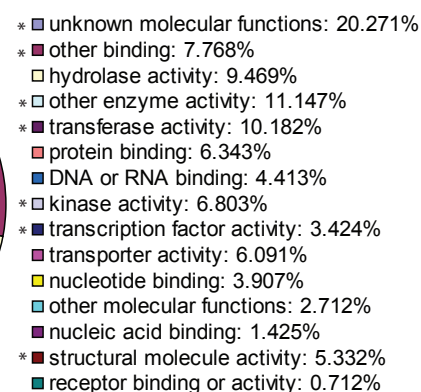
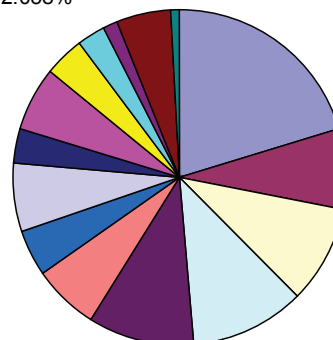
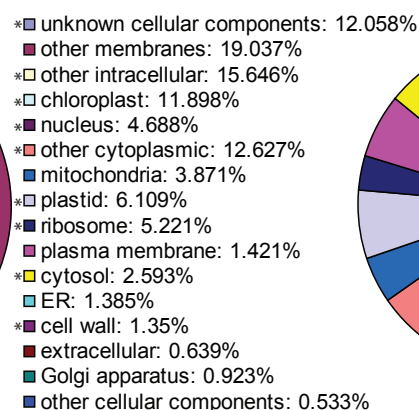
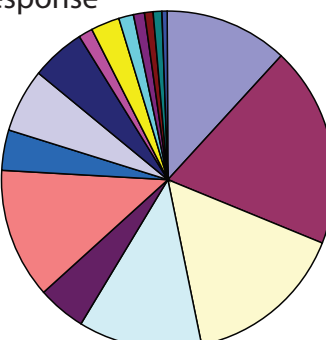
Downregulated under normal conditions



Upregulated in *aox1a* stress response

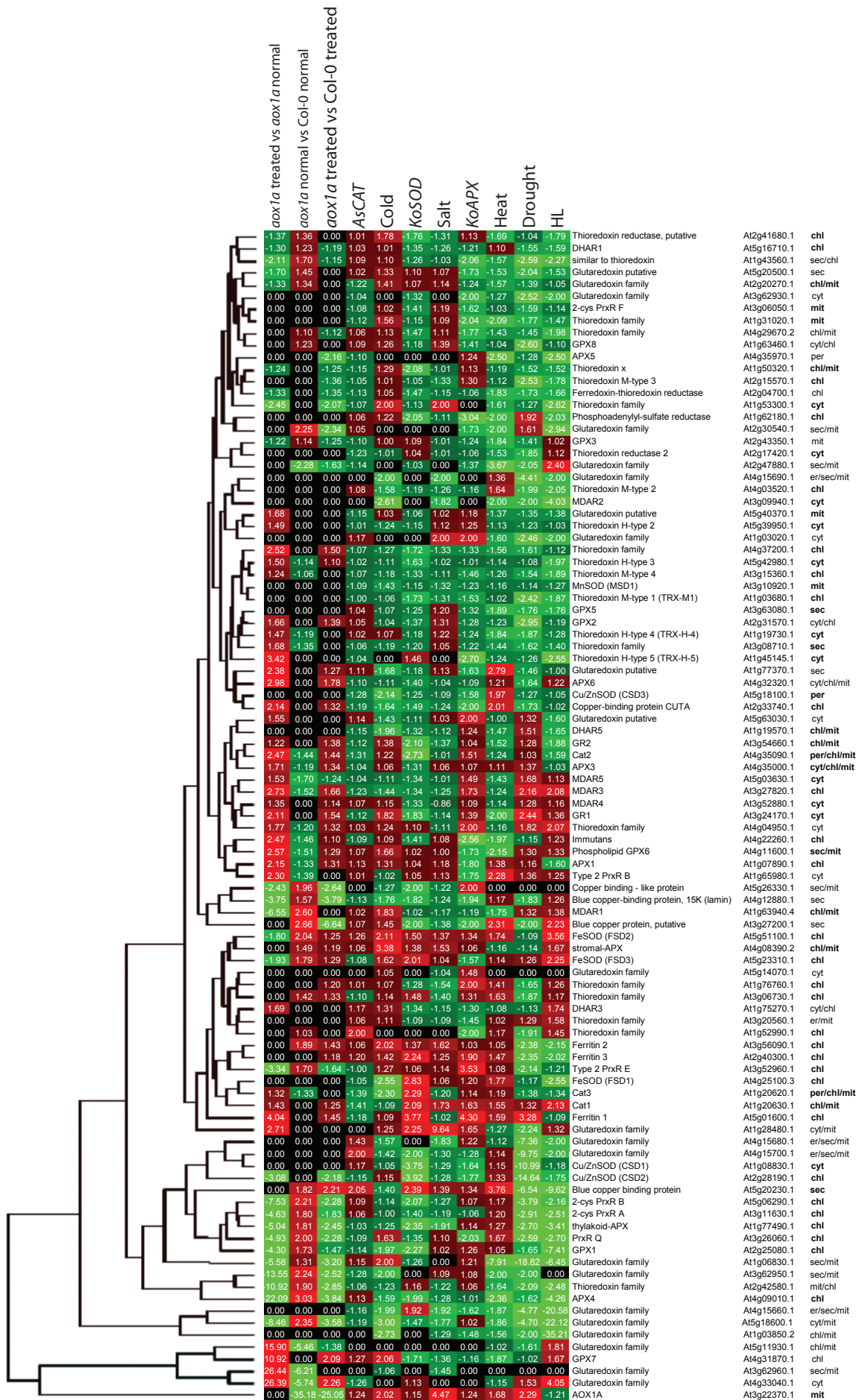
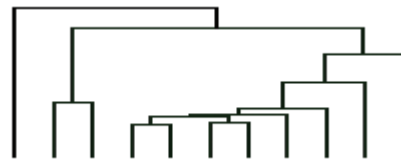


Downregulated in *aox1a* stress response

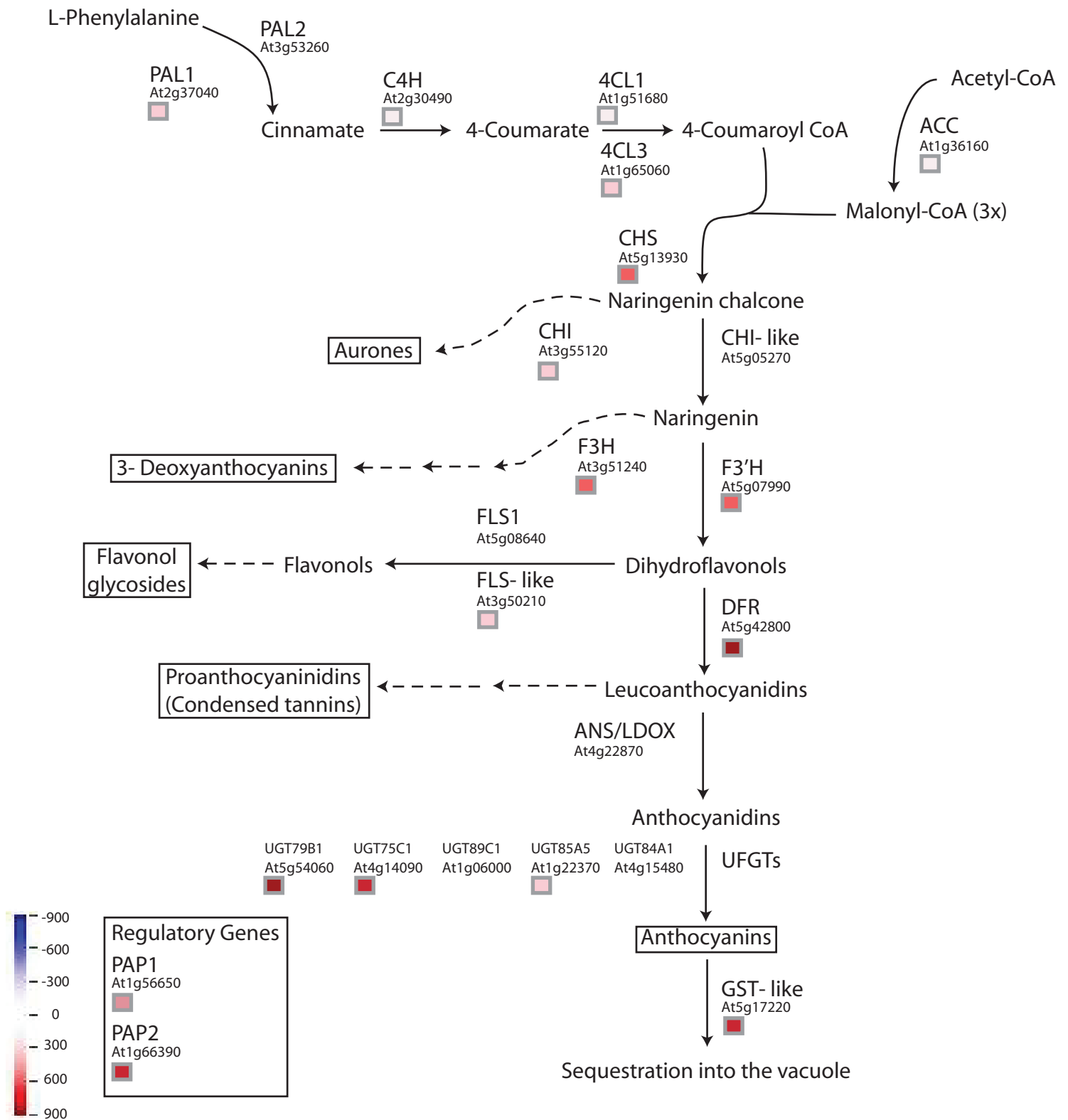


Supplementary Figure 3. Analysis of functional categorization of *aox1a* responsive transcripts.

Analysis of the changes in transcript abundance between Col-0 and *aox1a* under normal and combined drought and moderate light treatment. An asterisk indicates a significant difference as determined by Chi squared analysis between this set and the whole genome, a green asterisk indicates changes in this group are under-represented while a red asterisk indicates changes in this grouping are over-represented.



Supplementary Figure 4. Relative transcript abundance for genes encoding anti-oxidant defence components located in Arabidopsis. The relative expression data for anti-oxidant defence components for heat, drought, salt, cold, high-light treatments, knock-out superoxide dismutase, knock-out catalase and anti sense ascorbate peroxidase were taken from Mittler et al., 2004 and compared *aox1a* grown under normal conditions and *aox1a* under moderate light and drought treatments. Red indicated an increase and green a decrease in abundance, with numbers indicating magnitude on a linear scale, black indicates no significant change. Subcellular localisation was taken from Mittler et al. 2004.



Supplementary Figure 5. Changes in transcript abundance for genes that encode proteins involved in anthocyanin production. Anthocyanin production pathway was taken from Vanderauwera et al 2005 (Vanderauwera S, Zimmermann P, Rombauts S, Vandenabeele S, Langebartels C, Gruissem W, Inze D, Van Breusegem F (2005) Genome-wide analysis of hydrogen peroxide-regulated gene expression in Arabidopsis reveals a high light-induced transcriptional cluster involved in anthocyanin biosynthesis. *Plant Physiol* 139: 806-821). Changes in transcript abundance for *aox1a* untreated versus *aox1a* moderate light and drought treated is shown. Red indicates an up-regulation while blue indicates transcripts which are downregulated.